# OPTIMIZATION STUDIES OF LIQUID DIFFUSION INTERACTION FOR FABRICATION OF PAPER-BASED MICROFLUIDIC SENSING DEVICE

BY

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# ABSTRACT

Simple, low-cost and rapid sensing devices are in urgent demand for diagnostics purposes especially in resource-limited areas. Devices fabricated from patterning paper into hydrophilic and hydrophobic regions named as paper-based microfluidic analytical devices (μPADs) have been introduced. One of important parameter in the operation of μPADs is the fluid control. This project focused on the experimental and theoretical investigation of liquid flow rate analysis on μPADs. In the experimental part, the factors that affect the flow rate of liquid inside the channel have been investigated including the design of channel which is cut and wax channel, the platform of liquid flow which is in horizontal and vertical flow and the liquid viscosity. In the theoretical part, the pore size of paper have been investigated. In this project, straight line handcrafted channel at dimension of 3 mm width have been fabricated on 3 types of paper which is filter paper grade 1, chromatography paper grade 1 and blue litmus paper. 10 different viscosities of sucrose solutions ranging from 0 to 70 % of sucrose solute with the additional of 2 blind test samples have been tested on wax and cut channel with horizontal and vertical flow in a minute. The flow rate is then calculated using volumetric flow rate equation. In the theoretical part, the flow rate is calculated in MATLAB using Washburn equation and Darcy's law equation. In summary, it can be concluded that the horizontal liquid flow in cut channel have higher flow rate compared to vertical liquid flow in wax channel. Furthermore, high viscous liquid slow down the liquid flow in the channel. For the selection of paper, it is advisable to choose filter paper as the paper materials in the fabrication of μPADs compared to chromatography paper and litmus paper.

# خلاصة البحث

إن أجهزة الاستشعار البسيطة والرخيصة والسريعة مطلوبة بشكل عاجل للفحص، خاصة في المناطق المحدودة الموارد. تم في هذا البحث تقديم أداة مكونة من ورق شفاف عليها مناطق أليفة وطارئة للماء، سميت أداة الموائع الجزيئية التحليلية الورقية (μPADs)، ويعد التحكم في السوائل أحد العوامل المهمة عند استعمال الـ μPAD. ركز هذا المشروع على البحث التجريبي والنظري لتحليل معدل تدفق السائل في الـ μPAD. تم في الجزء التجريبي دراسة العوامل المؤثرة على معدل تدفق السائل داخل القناة، بما في ذلك التصميم، حيث صممت كقناة مقصوصة وقناة مشمعة، ومنصة تدفق السائل أفقيا ورأسيا، ولزوجة السائل. تم في الجزء النظري فحص حجم مسام الورق، وتم في هذا المشروع تصنيع القنوات يدويًا بخط مستقيم بعرض 3 مم على 3 أنواع من الورق وهي: ورق الترشيح من الدرجة الأولى، وورق الكروماتوغرافيا من الدرجة الأولى، وورق عباد الشمس الأزرق. تم اختبار 10 أزواج مختلفة لمحلول السكروز تراوحت بين 0 و 70٪ من محلول السكروز المذاب مع عينتين إضافيتين من عينات الاختبار المجهولة على القنوات المقصوصة والمشمعة بتدفق أفقي ورأسي في دقيقة واحدة. ثم تم حساب معدل التدفق باستخدام معادلة معدل التدفق الحجمي. في الجزء النظري تم حساب معدل التدفق في برنامج MATLAB باستخدام معادلة واشبورن ومعادلة قانون دارسي. باختصار، بالامكان استنتاج أن معدل التدفق الأفقي للسائل في القناة المقصوصة كان أعلى مقارنة بالتدفق العمودي للسائل في القناة المشمعة. بالإضافة إلى أن اللزوجة العالية للسائل تبطئ من تدفقه في القناة. لاختيار الورق ينصح باختيار ورق الترشيح لتصنيع الـ μPAD مقارنة بورق الكروماتوغرافيا وورق عباد الشمس.

# APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biosciences).

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# DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Nur Liyana binti Zamri

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*If the frontliners struggle battling with Covid 19*

*I struggle fighting with this thesis*

# ACKNOWLEDGEMENTS

Firstly, it is my utmost pleasure to dedicate this work to my dear parents and my family, who granted me the gift of their unwavering belief in my ability to accomplish this goal: thank you for your support and patience.

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# LIST OF SYMBOLS

| | |
|---|---|
| μPADs | Paper-based Microfludic Analytical Devices |
| Q | Flow rate |
| V | Volume of liquid |
| L | Path length of liquid flow |
| W | Width of channel |
| H | Thickness of paper |
| t | Time |
| γ | Surface tension |
| D | Pore diameter |
| μ | Viscosity of liquid |
| ΔP | Pressure difference |
| m | Mass of liquid |
| a | Acceleration of liquid |
| A | Area of channel |
| r | Accelerating length of liquid |
| π | Pi |
| Δv | Change in velocity |
| k | Permeability of paper |
| % | Percentage |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| WHO | World Health Organization |
| MEMS | Microelectromechanical Systems |
| PDMS | Polydimethylsiloxane |
| SEM | Scanning Electron Microscope |
| FP | Filter Paper |
| CP | Chromatography Paper |
| LP | Litmus Paper |
| VP | Vernier Callipers |
| PMMA | Polymethylmethacrylate |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE RESEARCH

Biosensor is an analytical device that undergo analysis of collecting, analyzing, observing and interpreting data that utilizes biological components to detect the biological analytes, together with a transducer to translate biological responses into electrical signals. It can be divided into 3 main components which are a recognition element, a signal transducer and a signal processor (Bohunicky & Mousa, 2011). The recognition elements that consist of enzymes, antibodies, proteins, antigens and nucleic acids detect the signal from the environment in the form of analytes (Bohunicky & Mousa, 2011). The signals then are converted by the transducer element into an electrical or digital signal that can be displayed, quantified and analyzed (Bohunicky & Mousa, 2011). According to Bohunicky and Mousa (2011), there are four general categories of transducers which are electrochemical, optical, calorimetric and mass changes. The basic features of a biosensor are linearity, sensitivity, selectivity and response time.

Biosensors have been applied in many fields including medical, agriculture and environment. In medicine, biosensors can be used for point of care and home health care such as monitoring blood glucose level for diabetes, diagnosing infectious diseases and detecting pathogens (Mehrotra, 2016). The biosensors are also used in agriculture field to increase the global food production, analyze the soil conditions and for agricultural monitoring (Rai et al., 2012). While in environment, the application of biosensors can be found in obtaining the clean water, clean air and healthy living (Rogers, 2006).

A type of biosensors that is significantly developing in this past few years is paper-based microfluidic sensors due to its low cost, simple fabrication, portable and disposable analytical devices that cover many application areas including biomedical, environmental monitoring and food quality control (Liana et al., 2012). Furthermore, because of its characteristics, the uses of paper-based sensors are highly demand especially in low resources area. This is compatible with the World Health Organization (WHO) needs where they mentioned that the diagnostics devices for developing countries should be ASSURED which are affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and deliverable to end-users (Martinez et al., 2009).

Microfluidics have been widely recognized as a new emerging technology in microanalytical systems (Nilghaz et al., 2012) as well as in sensors application. It is a field of research that uses small amount of liquids in micrometer scale channel to reduce the size and cost of fabrication, minimize the utilization of samples and reagents and address tiny objects like biological cells. In biosensors, microfluidics systems have been applied in paper-based sensors.

The pioneer microfluidic devices are fabricated from glass (Kotowski et al., 2013) and silicon (Keohane et al., 2014) since the fabrication techniques using these materials are well-developed (McDonald & Whitesides, 2002). Typically, glass micromachining and silica-based fabrication involves lithography procedures, dry and wet etching and specific techniques which require equipment and clean room facilities (Zhong et al., 2012). Furthermore, these techniques are time-consuming, expensive, need an access to specialized facilities and only marginally useful in research that require rapid evolution of prototypes (McDonald & Whitesides, 2002). Due to this, researchers have come out with new technology and materials for the fabrication of microfluidic devices.

A shift in this technology have been proven when microchannels are being fabricated using polymers which are polydimethylsiloxane (PDMS) (Fujii, 2002), polycarbonate (Wang et al., 2010) and polymethylmethacrylate (PMMA) (Holmes et al., 2011). This technology can reduce the production time and cost as well as eliminate the requirement of clean room facilities for the fabrication of microfluidic devices for various proof-of-concept demonstrations (Malon et al., 2017). However, fabrication using polymers need specialized equipment and integration of external components like pumps, valves and mixers to manipulate the fluid flow in the device as well as the need of employed trained personnel (Malon et al., 2017). Hence, the fabrication using other materials which is paper is fabricated to overcome these problems.

Paper has been used by human for centuries. They usually use it for writing, printing, drawing, filtering, packaging, cleaning and food wrapping but there are properties of paper that make it appropriate and significant for many other uses. The unique properties of paper have been utilized in the areas of technology like cell biology, electronics, robotics, microelectromechanical systems (MEMS) as well as microfluidic devices. According to Songok et al. (2014), paper is increasingly recognized nowadays as an interesting substrate in the production of paper-based microfluidic devices (μPADs) for possible applications in areas like health diagnosis, food safety, point-of-care and environmental monitoring.

Paper is a material which can be made thin, flexible and lightweight depending on its pulp processing (Liana et al., 2012). It is composed of cellulose fibre which made it highly attractive for certain applications that require the liquid penetration within its hydrophilic fibres matrix without the need of external equipment like pump and syringe

(Martinez et al., 2009). According to Hamedi et al. (2016), paper has been chosen as the platform in the fabrication of μPADs because of its porosity and hydrophilicity properties. Porosity causes immobilization of biomolecules inside the pores of paper while hydrophilicity allows the spontaneous wicking process of liquid which is useful for microfluidic application (Hamedi et al., 2016). In addition, paper is the cheapest material compared to silicon, glass and polymers hence it can be fabricated at low cost and budget. Instead of being portable and easy to operate (Liana et al., 2012), it is also safe to be used and can easily be incinerated for disposal (Hamedi et al., 2016).

### 1.1.1 Paper-based Microfluidic Analytical Devices (μPADs)

Paper-based Microfluidic Analytical Devices (μPADs) are fabricated by patterning paper into hydrophilic channels that are surrounded with hydrophobic barriers (Martinez, 2009). According to Martinez et al. (2007), the first research group that introduced μPADs are Whitesides research group in 2007 and have become an expanding research area until now.

The unique properties of μPADs that turning them into one of the interesting research area among the researchers are because of they are occupied with cellulose fibres that enable the capillary wicking of liquid along the channels. As the result, they do not require any pump or external sources of energy to generate the liquid flow in the channel (Parolo & Merkoci, 2013). Instead of low cost, they also can react in small amount of sample and can be used for multiplexed and quantitative analysis (Parolo & Merkoci, 2013).

μPADs are a promising platform that can be applied in various application areas like health diagnostics, food quality control and environmental monitoring. The main objectives of paper-based sensors is to provide a low-cost, easy-to-use, portable analytical platform for assays and affordable disease diagnosis and environmental monitoring for people living in developing world (Li et al., 2012).

In terms of application, μPADs can be functioning for on-demand devices, that involves a blank microfluidic platform without the immobilization of any indication reagents where the detection reagents can be chosen and tested by the users depending on the samples to be tested and ready-to-use devices, which means a complete sensors that work by integrating indication reagents to the detection zones for specific analytes detection in the samples (Li et al., 2012).

One of the important parameters in the operation of μPADs is the fluid control (Giokas et al., 2014). Fluid transport on paper is a passive process governed by capillary action and depends on the uniformity, homogeneity and physicochemical properties of paper, viscosity of fluid, dimensions of channel and environmental conditions (Giokas et al., 2014).

In this research which is to investigate the liquid diffusion interaction for the fabrication of paper-based microfluidic sensing device (μPAD), the main parameters that affect the liquid diffusion including the properties of paper, the design of the channel, the liquid sample and the liquid flow platform are investigated.

Hydrophilic channel is defined when the paper is drawn with hydrophobic barrier and heated for the diffusion of wax into the depth of paper. The width of channel are defined during the fabrication process while the height of channel is determined by the thickness of paper.

Three types of paper have been utilized in this research which are filter paper grade 1, chromatography paper grade 1 and blue litmus paper. The focus of using these types of paper is to investigate which paper have the best wicking properties that can wick the liquid inside the channel.

For the properties of paper, different pore sizes of paper have been studied using MATLAB simulation approach. While for the design of channel, the cut and wax boundaries of channel are introduced. Ten different viscosities of sucrose solution have been tested on the channel and for the liquid flow platform, lateral and vertical flow have been investigated.

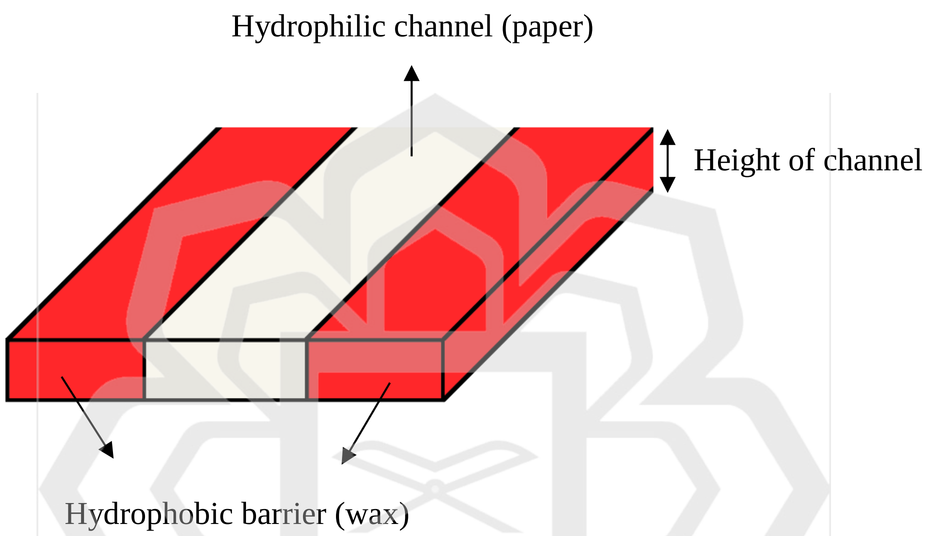


Figure 1.1 Cross Section of Hydrophilic Paper Surrounded with Hydrophobic Wax

Figure 1.1 illustrates the cross section of paper-based microfluidic device (μPAD) that is fabricated by drawing the hydrophobic barrier alongside the hydrophilic channel. The width of channel is defined during the fabrication process while the height of channel is defined by the thickness of the paper.

Carbohydrates consist of carbon, hydrogen and oxygen elements. The H : O ratio in all carbohydrate molecules is 2 : 1. There are 3 main types of carbohydrates which are monosaccharides, disaccharides and polysaccharides. Sucrose is one of the disaccharides. Sucrose is made up of glucose and fructose. It is found in sugar cane stems and sweet fruits. In this research, sucrose is chosen as the solute materials in the sample preparation for determining the effect of viscosity of samples on the flow rate of liquid inside the channel due to its ability of water-soluble and it is easier to get plus the cost is lower compared to glucose.

### 1.2 PROBLEM STATEMENT

Recently, the use of paper-based microfluidic analytical devices (μPADs) are really encouraging especially in diagnostics field due to its low-cost, easy-to-use, easy-to-handle, portable, user-friendly, can be used without supporting equipment but there are some important limitations that need to overcome.

Renault et al. (2013) stated that the liquid flow within cellulose channel was intrinsically slow. For example, it took around 20 minutes for water to flow through a 5 cm long of paper channel (Renault et al., 2013). These long analysis time might lead to problems like solvent evaporation (Renault et al., 2013). As for Camplisson et al. (2015), they agreed that time needed for the wicking of liquid inside the channel was one of the crucial characteristics in liquid flow mechanism. Thus, in order to overcome those problems, factors that affect the liquid flow in the cellulose channel is investigated throughout this research.

According to Songok and Toivakka (2016), flow rate on the paper channel was controlled by intrinsic properties of paper, porosity, permeability, liquid properties and channel size. Researchers have experimentally investigated and observed the effect of pore size on the flow rate of liquid by varying different pore sizes of cellulose materials. Shin et al. (2014) claimed that smaller pore size would decrease the flow rate. However, there is no validation on these statement from the researchers. Thus, the effect of pore sizes on the flow rate is studied using simulation approach.

One of the equation studied for the mechanism of liquid flow is Darcy's law. Based on the research done by Zhong et al. (2012), they assumed that the pressure difference inside the channel was constant. However, there is no concrete reason to prove those statement. Hence, in this research, a novel method of finding the value of pressure difference in Darcy's law equation is introduced.

Based on Giokas et al. (2014), they explained that the liquid flow on paper depend on the viscosity of liquid, dimensions of channel, properties of paper and environmental conditions. In order to overcome the limitation of μPADs in term of time for liquid flow, 10 different viscosities of sucrose solution is studied in this research. The aim is to know the effect of viscosity on the flow of liquid in the channel. Furthermore, for the dimensions of channel, cut and wax channel are applied throughout this study to relate the effect of dimensions of channel on the liquid flow rate. In addition, the direction of liquid flow either in horizontal and vertical flow is investigated to observe the effect of it on the rate of liquid flow in the paper channel.

## 1.3 RESEARCH OBJECTIVES

The main objective of this research is to investigate the factors that influence the liquid flow in the paper-based microfluidic analytical devices (μPADs) on three types of paper which are filter paper grade 1, chromatography paper grade 1 and blue litmus paper.

The specific objectives of this research are as follows:

i. To develop numerical simulation for analyzing the effect of pore size of paper on the flow rate of liquid using MATLAB.

ii. To measure the force and pressure of liquid on different viscosities of sucrose solution applied on the channel.

iii. To determine the factors that affect the flow rate of liquid in the μPADs channel:

   a. The effect of wax-cut channel and horizontal-vertical platform on the flow rate.

   b. The effect of viscosity of liquid on the flow rate and the validation using MATLAB simulation.

## 1.4 RESEARCH QUESTIONS

i. What happen to the flow rate of liquid in the channel when different pore sizes of paper are used?

ii. What is the effect of different viscosities of sucrose solution on the flow rate of liquid in the channel?

iii. Either wax or cut channel, which one can flow the highest flow rate of liquid in the channel?

iv. Either in horizontal or vertical liquid flow, which platform can flow the highest flow rate of liquid in the channel?

## 1.5 RESEARCH HYPOTHESIS

i. Larger diameter of pore size of paper increases the flow rate of liquid in the channel.

ii. The pressure on different viscosities of sucrose solution applied on the channel are obtained through experiments and calculations. Furthermore, more viscous solution slow down the flow of liquid in the channel.

iii. Cut channel increases the flow rate of liquid in the channel compared to wax channel.

iv. Horizontal platform increases the flow rate of liquid in the channel compared to vertical platform.

## 1.6 RESEARCH SCOPE AND LIMITATIONS

The fabrication technique applied in this research is simple and does not need the clean room environment. Furthermore, this research is conducted at room temperature thus, the humidity and temperature effect are negligible in the calculation. For the fabrication of channel, hand crafted technique is applied. Hence, the minimum width of channel that can be fabricated is 3 mm and this dimension is fixed throughout the experiment. For the investigation of force and pressure of liquid on different viscosities of sucrose solution applied on channel, the experiment is done on filter paper and chromatography paper only. Due to the fixed size of litmus paper produced from the manufacturer, the experiment done in this research cannot be tested on litmus paper. Hence, the force and pressure applied on litmus paper is considered as negligible throughout this experiment.

## 1.7 SIGNIFICANCE OF RESEARCH

i. **Low cost: The use of paper as the platform of μPAD reduces the cost**

   Paper have been used as the material for the fabrication of μPADs compared to silicon, glass and polymers due to its low cost, easy-to-get, user-friendly, can be incinerated for disposal and etc.

ii. **Easy fabrication: The use of paper ease the fabrication of μPADs**

   Fabrication process of μPADs does not need clean room facilities and supporting equipment like syringe and pump to travel the liquid inside the channel. Furthermore, by using hand-crafted fabrication technique, wax is drawn on the paper manually without the need of the wax printer.